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## **Genetic variants in the NOD2/CARD15 gene are associated with early mortality in sepsis patients**

Brenmoehl, Julia ; Herfarth, Hans ; Glück, Thomas ; Audebert, Franz ; Barlage, Stefan ; Schmitz, Gerd ; Froehlich, Dieter ; Schreiber, Stefan ; Hampe, Jochen ; Schölmerich, Jürgen ; Holler, Ernst ; Rogler, Gerhard

**Abstract:** Objective: Genetic variants in the NOD2/CARD15 gene resulting in diminished capacity to activate NF- $\kappa$ B in response to bacterial cell wall products have been associated with Crohn's disease (CD). Recently, we found an association between the variant Leu1007fsinsC of the NOD2/CARD15 gene (SNP13) and significantly increased rate of transplant related mortality (TRM) due to intestinal and pulmonary complications in stem cell transplantation (SCT). To assess a possible contribution of variants in the NOD2/CARD15 gene to sepsis related mortality (SRM) we investigated 132 prospectively characterised, consecutive patients with sepsis. Design and patients: The three most common NOD2/CARD15 variants (Arg702Trp, Gly908Arg, and Leu1007fsinsC) were determined in 132 prospectively characterised patients with sepsis attended to three intensive care units at the University of Regensburg by Taqman PCR. NOD2/CARD15 genotype and major patients' characteristics were correlated with SRM. Results: Patient groups with and without NOD2/CARD15 variants did not differ in their clinical characteristics such as median age, gender, reason for admission or APACHE score; however, SRM (day30) was increased in patients with NOD2/CARD15 coding variants (42 vs. 31%) and was highest (57%) in 8 patients carrying the Leu1007fsinsC variant ( $p < 0.05$ ). Multivariate analysis demonstrated the Leu1007fsinsC genetic variant as an independent risk factor for SRM. Conclusion: Our findings indicate a major role of NOD2/CARD15 coding variants for SRM. This may be indicative for a role of impaired barrier function and bacterial translocation in the pathophysiology of early sepsis related death

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**Abstract** *Objective:* Genetic variants in the NOD2/CARD15 gene resulting in a diminished capacity to activate NF- $\kappa$ B in response to bacterial cell wall products have been associated with Crohn's disease (CD). Recently, we found an association between the variant Leu1007fsinsC of the NOD2/CARD15 gene (SNP13) and a significantly increased rate of transplant related mortality (TRM) due to intestinal and pulmonary complications in stem cell transplantation (SCT). To assess a possible contribution of variants in the NOD2/CARD15 gene to sepsis related mortality (SRM) we investigated 132 prospectively characterised, consecutive patients with sepsis. *Design and patients:* The three most common NOD2/CARD15 variants (Arg702Trp, Gly908Arg, and Leu1007fsinsC) were determined

in 132 prospectively characterised patients with sepsis attended to three intensive care units at the University of Regensburg by Taqman PCR. NOD2/CARD15 genotype and major patients' characteristics were correlated with SRM. *Results:* Patient groups with and without NOD2/CARD15 variants did not differ in their clinical characteristics such as median age, gender, reason for admission or APACHE score; however, SRM (day 30) was increased in patients with NOD2/CARD15 coding variants (42 vs. 31%) and was highest (57%) in 8 patients carrying the Leu1007fsinsC variant ( $p < 0.05$ ). Multivariate analysis demonstrated the Leu1007fsinsC genetic variant as an independent risk factor for SRM. *Conclusion:* Our findings indicate a major role of NOD2/CARD15 coding variants for SRM. This may be indicative for a role of impaired barrier function and bacterial translocation in the pathophysiology of early sepsis related death.

**Keywords** Sepsis · NOD2/CARD15 · SNPs · Genetic risk factors

## Introduction

Sepsis is defined as the presence or presumed presence of an infection accompanied by evidence of a systemic response [1–3]. It is the most common cause of death in non-coronary intensive care units [4]. The current pathophysiological concepts of sepsis suggest a local immune response followed by a systemic release of pro-inflammatory and anti-inflammatory mediators, leading to a systemic imbalance of inflammation, progressive endothelial dysfunction, loss of blood pressure control, deterioration of the coagulation system and ultimately multiple organ dysfunctions [5–9].

Predisposing factors have been identified; among them patient age, pre-existing chronic diseases and immunosuppressive medication influence a patient's response to infection [10]. In recent years the role of genetic factors determining the susceptibility to sepsis and the severity of the disease has been explored [11–16]. A number of genetic polymorphisms have been identified influencing the risk of infection and/or of mortality. Single nucleotide polymorphisms (SNPs), as well as insertion and deletion polymorphisms, can characterize an individual's risk for sepsis, organ dysfunction or death: A polymorphism in the tumour necrosis factor (TNF)- $\alpha$  gene, the TNF-2 allele, has been associated with elevated serum levels of TNF and an increased risk of mortality from septic shock [13, 16, 17]. A SNP of the interleukin-1 receptor antagonist (IL-1ra) gene (IL-1RN\*2) has been associated with reduced IL-1ra production and consecutive increased mortality rates [18, 19]. Furthermore, polymorphisms in Toll-like receptor (TLR) and interferon- $\gamma$  genes also influence the susceptibility to sepsis [14, 16, 20].

The intestine is a reservoir of bacteria and of bacterial products (endotoxins, exotoxins and cell wall fragments) that may escape from the intestinal lumen to the mesenteric lymph nodes and the bloodstream. The intestinal mucosa is the major barrier against those bacteria and toxins, protecting the body from the potentially harmful pathogens. In sepsis the mucosal barrier is compromised and micro-organisms and their toxic products can gain access to the portal and systemic circulations with deleterious effects [21, 22]. After bacterial translocation across the mucosal barrier, systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) may develop leading to death of the patient [23]. Results of several clinical studies have demonstrated that bacteria isolated from patients with systemic infections are often the same strain as bacteria predominantly present in the fecal flora [24].

The mucosal barrier is formed primarily mechanically by the epithelial cell barrier and by intercellular junctions as well as immunologically the combined innate and adaptive immune system. Alterations in one these components of the intestinal barrier have been reported to be responsible for bacterial and toxin translocation. The cells of the

intestinal mucosa express a number of so-called pattern-recognition receptors (PRRs) which detect microbial components extra- or intra-cellularly [25, 26]. Mutations in a PRR, the NOD2/CARD15 gene, coding for a Caspase Recruitment Domain (CARD)-containing protein, were found in up to 40% of patients with Crohn's disease (CD), a chronic inflammatory bowel disease (IBD) [27–29]. Several SNPs in the NOD2/CARD15 gene have been identified, three of which have been shown to be independently associated with CD (Arg702Trp, Gly908Arg, and Leu1007fsinsC). Muramyl dipeptide (MDP), a component of the bacterial wall derived from peptidoglycane, is the ligand bound by NOD2 [30, 31]. The three major NOD2/CARD15 SNPs, Arg702Trp, Gly908Arg and Leu1007fsinsC, are all located within or near a leucine-rich repeat (LRR) region of NOD2/CARD15 [32]. The LRR domain is located at the C-terminus of the protein and represents the major structural motif that functions as a PRR for broad types of microbial components. In addition to the three major-risk alleles, a number of extremely rare amino acid polymorphisms, particularly within or near the LRR domain of NOD2/CARD15, have been defined.

Treatment of cells transfected with wild-type NOD2/CARD15 with bacterial peptidoglycan induces NF- $\kappa$ B activation. The frameshift variant, Leu1007fsinsC, truncates the LRR domain and is associated with a markedly reduced NF- $\kappa$ B activation [33, 34]. In comparison, the Arg702Trp and Gly908Arg variants respond more to stimulation with bacterial wall products than the Leu1007fsinsC variant; however, the ability to activate NF- $\kappa$ B is still significantly reduced.

NOD2/CARD15 is a member of a superfamily of genes, the NBS-LRR proteins (for nucleotide-binding site and leucine-rich repeat), which are involved in intracellular recognition of microbes and their products [35] and is expressed mainly in the Paneth cells of the epithelium as well as intestinal macrophages and dendritic cells. Mutations in NOD2/CARD15 also are a risk factor for the development of graft-vs.-host disease (GvHD) after allogeneic stem cell transplantation (SCT). Recently, we showed that the incidence of severe GvHD rose from 18% in donor/recipient pairs without any NOD2 mutation to 37% in pairs with either donor or recipient mutations with a subsequent increase of treatment related mortality (TRM) from 33 to 60% [36–38]. When donor and recipient both had NOD2 mutations severe GvHD rose from 22 to 55% and TRM from 38 to 100% [36–38]. A deficient antibacterial response in Paneth cells of the recipient donor monocytes could result in increased bacterial translocation [34, 39–41]. We could further demonstrate that TRM in patients with Arg702Trp, Gly908Arg and Leu1007fsinsC mutations was caused mainly by intestinal and pulmonary complications, which were likely due to bacterial translocation in these organs [38]. This led us to investigate

the role of NOD2/CARD15 mutations in patients with sepsis [42].

## Material and methods

### Patients

Approval for this study was obtained from the local institutional review board. All patients who were admitted to the intensive care units of the University Hospital of Regensburg between 2001 and April 2004 were evaluated prospectively for their clinical data. One hundred thirty-two patients were included in the study when they fulfilled the criteria of sepsis. According to the Consensus Conference Criteria [43], sepsis was diagnosed based on the presence of at least three systemic inflammatory response syndrome criteria with the simultaneous identification of focal infection by clinical, radiological or microbiological criteria. Microbiological cultures were done according to standard techniques. Sepsis-related mortality was defined as mortality within 30 days after study entry, as obtained from hospital records. The Acute Physiology and Chronic Health Evaluation (APACHE) [44–47] and the Simplified Acute Physiology Score II (SAPS II) [48, 49] were used to classify the severity of sepsis. The first blood sample was drawn as soon as possible after patients fulfilled the criteria of sepsis for the first time (day 1).

All DNA studies were carried out after the patients were clinically characterized and had been entered into the database.

### Isolation of DNA

The DNA was isolated from buffy coats of EDTA blood samples with DNeasy columns (Quiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA was precipitated by adding 100% isopropanol. The supernatant was carefully removed, the pellet air-dried and re-suspended in 50–200 µL sterile water. The DNA-concentration was measured with 1:20 pre-diluted genomic DNA at 260 and 280 nm wavelength. The isolated genomic DNA was diluted to a final concentration of 20 ng/µL and stored at –20 °C.

### Determination of NOD2 genotype

Single nucleotide polymorphisms Arg702Trp (SNP8), Gly908Arg (SNP12) and Leu1007fsinsC (SNP13) were

determined by allele specific PCR (Taqman). A 10-µL reaction mix for one SNP-typing consisted of 5 µL of 2xTaqMan Universal PCR Master Mix (Applied Biosystems), 1 µL of forward and reverse primer, 1 µL of wildtype- and mutant probe and 20 ng genomic DNA.

All reactions were performed in 384-well plates (Abgene, Epsom, UK) in an external thermocycler (Primus-HT; MWG Biotech). The cyclor conditions were: 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s (melting) and 60 °C for 1 min (annealing/extension). During the PCR the released fluorescence was measured by the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Forster City, Calif.)

Increases in the amount of reporter dye fluorescence during the 45 cycles of amplification were analysed using Sequence Detector software (SDS version 2.2, PE Applied Biosystems). Changes in reporter dye fluorescence of 6-FAM vs. changes in reporter dye fluorescence of TET were plotted (homozygous FAM vs. homozygous TET vs. heterozygous FAM/TET). [For primer and PCR probe (all synthesized by MWG Biotech, Ebersberg, Germany) sequences see ESM].

A total of 117 DNA samples were additionally completely sequenced to confirm Taqman results and to detect private mutations in the relevant exons. In the remaining 15 patients no complete sequence could be obtained. All together, five private mutations were found. All of them were silent mutations not associated with changes in the amino acid sequence.

### Statistics

Major patient characteristics, such as age, gender, category of admission (surgical emergency/other), underlying disease (malignant/haematological vs. others) as well as the results of NOD2/CARD15 genotyping, were listed for each patient. Haematological patients were regarded as separate group due to their bad outcome [50]. Patient characteristics, APACHE and SAPS scores at admission and in the course of ICU treatment as well as outcome (survival at day 30) were analysed in relation to NOD2/CARD15 mutations. Frequencies were compared by chi-square analysis, and *t*-tests were used for parametric variables. Survival was calculated using Kaplan–Meier statistics, and groups were compared by log-rank tests. For multivariate analysis of risk factors affecting survival and confounding variables, such as age and gender, stepwise Cox regression models were used (Table 1). The limit for reverse selection process was 0.2. Statistical analysis was performed by the help of the SPSS software (version 12.00).

**Table 1** Multivariate Cox regression analysis of 30-day survival. *HR* hazard ratio with 95% CI are shown. The following groups were compared with regard to NOD2/CARD15 mutations: WT vs. SNP8 and 12 (Arg702Trp/Gly908Arg) vs. SNP 13 (Leu1007fsinsC)

Parameter	Group	Survival (Kaplan–Meier, %)	Significance ( <i>p</i> )	HR	95% CI
Age (years)	< 60	69	n.s.	1.16	0.5–2.5
	> 60	61			
Category of admission	Other	69	n.s.	1.89	0.9–3.9
	Surgical emergency	58			
Underlying disease	Non-haematological	72	n.s.	1.72	0.6–4.3
	haematological/malignancy	64			
NOD2/CARD15	Wildtype	68.9	n.s.	1.72	0.61.4–10.14.7
Mutation	Arg702Trp/Gly908Arg	64.7/37.5	0.027	3.72	1.4–10.1
	Leu1007fsinsC				

## Results

Patients' characteristics of the groups with wildtype (WT) and variant NOD2/CARD15 sequence did not differ significantly (see Table 2). The median age of the 107 WT patients was 55 years (range 20–91 years) of the 17 Arg702Trp or Gly908Arg (Arg702Trp/Gly908Arg) mutated patients 70 years (range 17–80 years) and of the 8 patients with Leu1007fsinsC 58 years (range 35–93 years). Of the 17 patients of the Arg702Trp/Gly908Arg group, only 2 had the Gly908Arg variant. No homozygous or compound heterozygous patients with NOD2/CARD15 variants were among our patients.

The gender ratio (female/male) was WT: 33/74, Arg702Trp/Gly908Arg: 2/15 and Leu1007fsinsC: 3/5. Emergency surgery as the reason for admission occurred in 33 (31%), 2 (12%) and 3 (38%) in the respective groups. Malignancy as underlying disease was present in 19 (18%) of WT, 3 (18%) of Arg702Trp/Gly908Arg and 0 of Leu1007fsinsC patients. Previous surgery had been performed in 48 (45%) WT, 5 (29%) Arg702Trp/Gly908Arg and 2 (25%) Leu1007fsinsC patients.

Gram-positive bacteria were isolated from blood samples in 29 (27%) WT patients, 5 (29%) Arg702Trp/Gly908Arg patients and 4 (50%) Leu1007fsinsC individuals (see Table 2). The respective number for Gram-negative bacteria were 25 (23%), 5 (29%) and 4 (50%). This indicates that in 50% of all patients without

NOD2/CARD15 variants, 58% of all individuals with Arg702Trp or Gly908Arg variants (n.s. vs. WT) and 100% of Leu1007fsinsC variant patients ( $p < 0.05$  vs. WT,  $z = 2.3$ , chi-square test), Gram-negative or Gram-positive bacteria could be detected in blood cultures. Fungi, viruses or mixed infections were found in 34 (32%) WT patients, 2 (12%) Arg702Trp/Gly908Arg patients and in none of the Leu1007fsinsC patients.

As rated by the treating doctors the infectious focus was likely to be pulmonary in 45 (42%) of NOD2/CARD15 WT patients, 9 (53%) Arg702Trp/Gly908Arg patients and 5 (63%) Leu1007fsinsC individuals (see Table 2). The respective numbers for the gastrointestinal tract to be the most likely focus of sepsis were 30 (28%), 1 (6%) and 2 (25%).

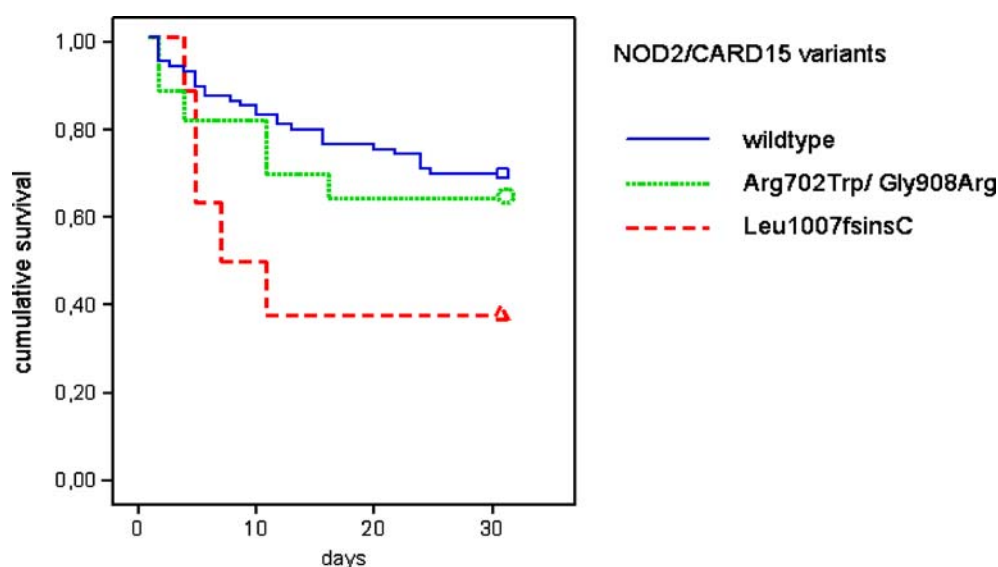
Whereas there were no differences in functional parameters at admission, there were significant differences after 1 week (Table 2). At admission APACHE scores were (mean  $\pm$  SD)  $22.6 \pm 6.9$  in WT,  $20.5 \pm 7.7$  in Arg702Trp/Gly908Arg and  $23.1 \pm 5.3$  in Leu1007fsinsC patients. The SAPS scores were  $45.2 \pm 15.3$ ,  $43.9 \pm 18$  and  $48.6 \pm 9.4$ , and  $\text{FiO}_2$  as a parameter of respiratory function was  $0.50 \pm 0.19$ ,  $0.47 \pm 0.19$  and  $0.46 \pm 0.20$ , respectively. After 1 week, APACHE scores were  $19.6 \pm 7.1$  in WT,  $20.0 \pm 7.6$  in Arg702Trp/Gly908Arg and  $21.5 \pm 11.4$  in Leu1007fsinsC patients ( $p = 0.08$ ). The SAPS scores were  $40.7 \pm 17.8$ ,  $42.0 \pm 15.0$  and  $44.1 \pm 18.0$ , respectively (n.s.).  $\text{FiO}_2$  was  $0.40 \pm 0.14$  in

**Table 2** Characteristics of the patient populations with respect to disease severity. WT wildtype

Parameter	WT patients	Arg702Trp/Gly908Arg	Leu1007fsinsC
APACHE score at admission	$22.6 \pm 6.9$	$20.5 \pm 7.7$	$23.1 \pm 5.3$
SAPS score at admission	$45.2 \pm 15.3$	$43.9 \pm 18$	$48.6 \pm 9.4$
$\text{FiO}_2$ at admission	$0.50 \pm 0.19$	$0.47 \pm 0.19$	$0.46 \pm 0.20$
$\text{pO}_2/\text{FiO}_2 < 200$ at admission ( <i>n</i> )	70 (66)	11 (64)	6 (75)
APACHE score at week1	$19.6 \pm 7.1$	$20.0 \pm 7.6$	$21.5 \pm 11.4$
SAPS score at week1	$40.7 \pm 17.8$	$42.0 \pm 15.0$	$44.1 \pm 18.0$
$\text{FiO}_2$ at week1	$0.40 \pm 0.14$	$0.45 \pm 0.23$	$0.54 \pm 0.31$
			( $p < 0.001$ vs. WT)
$\text{pO}_2/\text{FiO}_2 < 200$ week 1 ( <i>n</i> )	41 (42)	7 (41)	5 (63)

Numbers in parentheses are percentages

**Fig. 1** Kaplan–Meier survival plot of sepsis patients grouped according to presence or absence of NOD2/CARD15 Arg702Trp/Gly908Arg or Leu1007fsinsC mutations. Differences between groups were compared by log-rank analysis



WT,  $0.45 \pm 0.23$  in Arg702Trp/Gly908Arg ( $p < 0.01$  vs. WT) and  $0.54 \pm 0.31$  ( $p < 0.001$  vs. WT).

Sepsis-related mortality after 18 days was 19% in WT, 29% in Arg702Trp/Gly908Arg and 57% in Leu1007fsinsC patients. After 31 days respective data were 31, 36 and 57%. Kaplan–Meier survival plot of sepsis patients grouped according to presence or absence of NOD2/CARD15 Arg702Trp/Gly908Arg or Leu1007fsinsC mutations is given in Fig. 1. Differences between groups were compared by log-rank analysis. A significantly increased risk for SRM was evident in patients with Leu1007fsinsC ( $p = 0.03$ ).

Multivariate analysis clearly demonstrated Leu1007fsinsC to be an independent risk factor for early SRM (Table 2; HR = 3.72, 95% CI = 1.4–10.1;  $p = 0.027$ ).

## Discussion

Our study is the first to report a strong impact of a NOD2/CARD15 variant (Leu1007fsinsC) on sepsis-related mortality (SRM) and the outcome of sepsis. The frequency of NOD2/CARD15 variants among patients with sepsis was not found to be increased compared with healthy control populations as reported by others. Among 132 patients 25 individuals carrying NOD2/CARD15 variants were detected corresponding to 19% of the investigated cohort. The reported frequency of heterozygous NOD2/CARD15 variants among healthy German patients in a study on CD was 14% [29]. In an own study in a stem cell transplantation setting NOD2/CARD15 mutations occurred with a frequency of 21.8% in patients and 13.7% in stem cell donors [36]. In the latter study only one homozygous mutation was observed, whereas all other patients and donors with mutations were heterozygous.

This indicates that the frequency of NOD2/CARD15 variants is not increased in sepsis patients and NOD2 mutations are not a susceptibility factor for sepsis.

As the Leu1007fsinsC had the most impact in the mentioned study in stem cell transplantation [36], we compared three groups: Sepsis patients with WT NOD2/CARD15, patients carrying the Arg702Trp or Gly908Arg and individuals with the Leu1007fsinsC variant. The patient groups did not differ in their baseline characteristics indicating that NOD2/CARD15 variants do not provoke early “spontaneous” sepsis (Table 3). Comorbidity did not seem to play a major role; however, the pattern of microbiological causes of sepsis (bacteria, fungi, viruses or idiopathic) was different between the groups. Gram-positive or Gram-negative bacteria seemed to play the most prominent role in patients with the Leu1007fsinsC variant. Only in 50% of the WT patients bacteria could be grown from blood samples in contrast to 58% of all individuals with Arg702Trp or Gly908Arg variants and 100% of Leu1007fsinsC variant patients.

Whereas severity of sepsis as indicated by APACHE and SAPS scores was not different at admission, there were significant differences in APACHE score and FiO<sub>2</sub> as well as a trend to a higher number of patients with a pO<sub>2</sub>/FiO<sub>2</sub> ratio < 200 in the Leu1007fsinsC patients indicating functional relevance of NOD2/CARD15 variants for sepsis outcome. A PaO<sub>2</sub>/FiO<sub>2</sub> ratio below 200 mmHg is one of the diagnostic criteria of ARDS according to the American–European Consensus Conference, yet this ratio is affected by a number of factors such as ventilator settings and FiO<sub>2</sub> per se [51]. The most important finding, however, was the impact of the Leu1007fsinsC variant on sepsis-related mortality which was clearly visible in Kaplan–Meier survival plot and log-rank analysis. Multivariate analysis further demonstrated Leu1007fsinsC

**Table 3** Baseline characteristics of the patient populations

Parameter	WT patients	Arg702Trp/Gly908Arg	Leu1007fsinsC
Age (years)	55 (range 20–91)	70 (range 17–80)	58 (range 35–93)
Gender (female/male)	33/74	2/15	3/5
Emergency surgery ( <i>n</i> )	33 (31)	2 (12)	3 (38)
Malignancy as underlying disease ( <i>n</i> )	19 (18)	3 (18)	0
Previous surgery ( <i>n</i> )	48 (45)	5 (29)	2 (25)
Gram-positive bacteria found ( <i>n</i> )	29 (27)	5 (29)	4 (50)
Gram-negative bacteria found ( <i>n</i> )	25 (23)	5 (29)	4 (50)
Fungi, viruses or mixed infections ( <i>n</i> )	34 (32)	2 (12)	0
Infectious focus likely to be pulmonary	45 (42)	9 (53)	5 (63)
Infectious focus likely to be intestinal	30 (28)	1 (6)	2 (25)
Pulmonary comorbidity (e.g. COLD, %)	17	41	0
Diabetes (%)	14	0	14
Severe cardiovascular comorbidity (%)	9	0	0

The focus of infection was suggested by the treating physician to be the most likely  
Numbers in parentheses are percentages

to be an independent risk factor for early SRM. This is in line with our finding in stem cells transplantation in which we also found a major influence of especially the Leu1007fsinsC variant: Overall, severe GvHD, severe gastrointestinal GvHD and transplantation related mortality (TRM) at 1 year were significantly increased in recipient/donor pairs with NOD2/CARD15 mutations as compared with the WT group [36].

The strong association of TRM with NOD2/CARD15 mutations was partially explained by an increased risk not only for death from GvHD, but also from respiratory failure due to diffuse primary or secondary pulmonary damage. Whereas in the WT group only 11 of 30 (37%) deaths in remission were caused by GvHD and respiratory failure, the proportion increased to 17 of 26 (65%) in pairs with NOD2/CARD15 mutations [36]. In further analysis by multivariate Cox regression for TRM in the matched unrelated donor (MUD) cohort (*n*=342) the Leu1007fsinsC variant was the only independent risk factor identified with a HR of 2.9 (95% CI 1.5–5.5, *p* < 0.001) when recipient age, match or mismatch, stage of underlying disease, use of T-cell depletion and intensity of conditioning were used as covariates.

As the Leu1007fsinsC mutation has been reported to impair detection of bacterial translocation and invasion in the mucosa, our findings may also have implications for the pathophysiological concept of sepsis: A contribution of the intestinal barrier to the risk and the disease course of sepsis has long been discussed. Intestinal permeability seems to be a factor strongly determining the outcome of sepsis [52–56]. Bacterial translocation not only occurs in animal models but also in humans: In patients with fatal burns more than 50% have intestinal lesions and more than 80% are septic—mostly with intestinal organisms [57]. The association between increased gut permeability and infectious complications, however, is controversial. So far, no causative direct link between gut barrier dysfunction

and infectious complications in humans could be identified. Our study provides the first evidence for a mechanism that could be involved.

The NOD2/CARD15 protein plays a major role in the intra-cytoplasmatic response to bacterial cell wall products and subsequent NF- $\kappa$ B activation in monocytes/macrophages and epithelial cells [58]. The association of NOD2/CARD15 mutations with SRM suggests a predominant role of an impaired monocyte/macrophage response or an impaired epithelial barrier resulting in dysregulation of inflammation. The exact mechanisms of diminished monocyte/macrophage response, impaired barrier function and the imbalance of inflammatory responses in sepsis remain to be elucidated; however, our findings indicate that the translocation of intestinal microflora may directly interfere with immune cell activation and should stimulate further investigations.

In stem cell transplantation a gut–liver–lung–axis of impaired elimination of intestinal LPS by the liver has been postulated to be involved in development of pulmonary damage [38, 59]. Direct involvement of an impaired monocyte/macrophage response in the lung, however, might be an alternative explanation and should be addressed in careful analyses in future studies in SCT as well as in sepsis.

In conclusion, our data suggest a role of variants of NOD2/CARD15 gene as a new genetic factor determining the outcome of sepsis in a subgroup of patients. As discussed for CD and GvHD, consideration of sepsis mortality as a result of epithelial barrier and/or monocyte/macrophage defect resulting in impaired response to bacteria could influence the pathophysiological concepts.

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## References

- Calandra T, Cohen J (2005) The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 33:1538–1548
- Jacobi J (2002) Pathophysiology of sepsis. *Am J Health Syst Pharm* 59(Suppl 1):S3–S8
- Abraham E, Matthay MA, Dinarello CA, Vincent JL, Cohen J, Opal SM, Glauser M, Parsons P, Fisher CJ Jr, Repine JE (2000) Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: time for a reevaluation. *Crit Care Med* 28:232–235
- Gluck T, Opal SM (2004) Advances in sepsis therapy. *Drugs* 64:837–859
- Liu SF, Malik AB (2006) NF- $\kappa$ B activation as a pathological mechanism of septic shock and inflammation. *Am J Physiol Lung Cell Mol Physiol* 290:L622–L645
- Kobayashi M, Tsuda Y, Yoshida T, Takeuchi D, Utsunomiya T, Takahashi H, Suzuki F (2006) Bacterial sepsis and chemokines. *Curr Drug Targets* 7:119–134
- Hoesel LM, Gao H, Ward PA (2006) New insights into cellular mechanisms during sepsis. *Immunol Res* 34:133–141
- Johnson GB, Brunn GJ, Samstein B, Platt JL (2005) New insight into the pathogenesis of sepsis and the sepsis syndrome. *Surgery* 137:393–395
- Cavaillon JM, Adib-Conquy M (2005) Monocytes/macrophages and sepsis. *Crit Care Med* 33:S506–S509
- Angus DC, Wax RS (2001) Epidemiology of sepsis: an update. *Crit Care Med* 29:S109–S116
- Tabrizi AR, Zehnbauser BA, Freeman BD, Buchman TG (2001) Genetic markers in sepsis. *J Am Coll Surg* 192:106–117; quiz 145–146
- Sipahi T, Pocan H, Akar N (2006) Effect of various genetic polymorphisms on the incidence and outcome of severe sepsis. *Clin Appl Thromb Hemost* 12:47–54
- Dahmer MK, Randolph A, Vitali S, Quasney MW (2005) Genetic polymorphisms in sepsis. *Pediatr Crit Care Med* 6:S61–S73
- Imahara SD, O'Keefe GE (2004) Genetic determinants of the inflammatory response. *Curr Opin Crit Care* 10:318–324
- Wunderink RG, Waterer GW (2003) Genetics of sepsis and pneumonia. *Curr Opin Crit Care* 9:384–389
- Holmes CL, Russell JA, Walley KR (2003) Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. *Chest* 124:1103–1115
- Gordon AC, Lagan AL, Aganna E, Cheung L, Peters CJ, McDermott MF, Millo JL, Welsh KI, Holloway P, Hitman GA, Piper RD, Garrard CS, Hinds CJ (2004) TNF and TNFR polymorphisms in severe sepsis and septic shock: a prospective multicentre study. *Genes Immun* 5:631–640
- Fang XM, Schroder S, Hoeft A, Stuber F (1999) Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis. *Crit Care Med* 27:1330–1334
- Arnalich F, Lopez-Maderuelo D, Codoceo R, Lopez J, Solis-Garrido LM, Capiscol C, Fernandez-Capitan C, Madero R, Montiel C (2002) Interleukin-1 receptor antagonist gene polymorphism and mortality in patients with severe sepsis. *Clin Exp Immunol* 127:331–336
- Stassen NA, Leslie-Norfleet LA, Robertson AM, Eichenberger MR, Polk HC Jr (2002) Interferon-gamma gene polymorphisms and the development of sepsis in patients with trauma. *Surgery* 132:289–292
- Fink MP (2003) Intestinal epithelial hyperpermeability: update on the pathogenesis of gut mucosal barrier dysfunction in critical illness. *Curr Opin Crit Care* 9:143–151
- Rowlands BJ, Soong CV, Gardiner KR (1999) The gastrointestinal tract as a barrier in sepsis. *Br Med Bull* 55:196–211
- Souza DA de, Greene LJ (2005) Intestinal permeability and systemic infections in critically ill patients: effect of glutamine. *Crit Care Med* 33:1125–1135
- Wells CL, Juni BA, Cameron SB, Mason KR, Dunn DL, Ferrieri P, Rhame FS (1995) Stool carriage, clinical isolation, and mortality during an outbreak of vancomycin-resistant enterococci in hospitalized medical and/or surgical patients. *Clin Infect Dis* 21:45–50
- Forchielli ML, Walker WA (2005) The role of gut-associated lymphoid tissues and mucosal defence. *Br J Nutr* 93(Suppl 1):S41–S48
- Yuan Q, Walker WA (2004) Innate immunity of the gut: mucosal defense in health and disease. *J Pediatr Gastroenterol Nutr* 38:463–473
- Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gas-sull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411:599–603
- Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411:603–606
- Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeier A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG (2001) Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 357:1925–1928
- Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 278:5509–5512
- Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 278:8869–8872
- Bonen DK, Cho JH (2003) The genetics of inflammatory bowel disease. *Gastroenterology* 124:521–536
- Barnich N, Aguirre JE, Reinecker HC, Xavier R, Podolsky DK (2005) Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor- $\kappa$ B activation in muramyl dipeptide recognition. *J Cell Biol* 170:21–26
- Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G (2001) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF- $\kappa$ B. *J Biol Chem* 276:4812–4818
- Chamaillard M, Girardin SE, Viala J, Philpott DJ (2003) Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell Microbiol* 5:581–592



36. Holler E, Rogler G, Herfarth H, Brenmoehl J, Wild PJ, Hahn J, Eissner G, Scholmerich J, Andreesen R (2004) Both donor and recipient NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. *Blood* 104:889–894
37. Rogler G, Holler E (2004) Can NOD2/CARD15 mutations predict intestinal graft-versus-host disease and aid our understanding of Crohn's disease? *Nat Clin Pract Gastroenterol Hepatol* 1:62–63
38. Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H, Dickinson AM, Socie G, Wolff D, Fischer G, Jackson G, Rocha V, Steiner B, Eissner G, Marienhagen J, Scholmerich J, Andreesen R (2006) Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. *Blood* 107:4189–4193
39. Gutierrez O, Pipaon C, Inohara N, Fontalba A, Ogura Y, Prosper F, Nunez G, Fernandez-Luna JL (2002) Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor- $\kappa$ B activation. *J Biol Chem* 277:41701–4175
40. Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, Nunez G (2003) Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 52:1591–1597
41. Eckmann L (2004) Innate immunity and mucosal bacterial interactions in the intestine. *Curr Opin Gastroenterol* 20:82–88
42. Brenmoehl J, Herfarth H, Glück T, Audebert F, Barlage S, Schmitz G, Froehlich D, Schreiber S, Hampe J, Schölmerich J, Holler E, Rogler G (2005) Genetic variants in the NOD2/CARD15 gene are associated with early mortality in sepsis patients. *Gastroenterology* 128:T1020 (abstract)
43. Bone RC, Sprung CL, Sibbald WJ (1992) Definitions for sepsis and organ failure. *Crit Care Med* 20:724–726
44. Goldhill DR, Sumner A (1998) APACHE II, data accuracy and outcome prediction. *Anaesthesia* 53:937–943
45. Hurr H, Hawley HB, Czachor JS, Markert RJ, McCarthy MC (1999) APACHE II and ISS scores as predictors of nosocomial infections in trauma patients. *Am J Infect Control* 27:79–83
46. Lockrem JD, Lopez E, Gallagher J, Church GE, Estafanous FG (1991) Severity of illness: APACHE II analysis of an ICU population. *Cleve Clin J Med* 58:477–486
47. Taylor SL, Morgan DL, Denson KD, Lane MM, Pennington LR (2005) A comparison of the Ranson, Glasgow, and APACHE II scoring systems to a multiple organ system score in predicting patient outcome in pancreatitis. *Am J Surg* 189:219–222
48. McNelis J, Marini C, Kalimi R, Jurkiewicz A, Ritter G, Nathan I (2001) A comparison of predictive outcomes of APACHE II and SAPS II in a surgical intensive care unit. *Am J Med Qual* 16:161–165
49. Aegerter P, Boumendil A, Retbi A, Minvielle E, Dervaux B, Guidet B (2005) SAPS II revisited. *Intensive Care Med* 31:416–423
50. Andrews P, Azoulay E, Antonelli M, Brochard L, Brun-Buisson C, Dobb G, Fagon JY, Gerlach H, Groeneveld J, Mancebo J, Metnitz P, Nava S, Pugin J, Pinsky M, Radermacher P, Richard C, Tasker R (2006) Year in review in intensive care medicine, 2005. II. Infection and sepsis, ventilator-associated pneumonia, ethics, haematology and haemostasis, ICU organisation and scoring, brain injury. *Intensive Care Med* 32:380–390
51. Andrews P, Azoulay E, Antonelli M, Brochard L, Brun-Buisson C, Dobb G, Fagon JY, Gerlach H, Groeneveld J, Mancebo J, Metnitz P, Nava S, Pugin J, Pinsky M, Radermacher P, Richard C, Tasker R, Vallet B (2005) Year in review in intensive care medicine, 2004. I. Respiratory failure, infection, and sepsis. *Intensive Care Med* 31:28–40
52. Andrews P, Azoulay E, Antonelli M, Brochard L, Brun-Buisson C, Dobb G, Fagon JY, Gerlach H, Groeneveld J, Mancebo J, Metnitz P, Nava S, Pugin J, Pinsky M, Radermacher P, Richard C, Tasker R, Vallet B (2005) Year in review in intensive care medicine, 2004. III. Outcome, ICU organisation, scoring, quality of life, ethics, psychological problems and communication in the ICU, immunity and hemodynamics during sepsis, pediatric and neonatal critical care, experimental studies. *Intensive Care Med* 31:356–372
53. Brooks SG, May J, Sedman P, Tring I, Johnstone D, Mitchell CJ, MacFie J (1993) Translocation of enteric bacteria in humans. *Br J Surg* 80:901–902
54. Sedman PC, Macfie J, Sagar P, Mitchell CJ, May J, Mancey-Jones B, Johnstone D (1994) The prevalence of gut translocation in humans. *Gastroenterology* 107:643–649
55. D'Amico R, Pifferi S, Leonetti C, Torri V, Tinazzi A, Liberati A (1998) Effectiveness of antibiotic prophylaxis in critically ill adult patients: systematic review of randomised controlled trials. *Br Med J* 316:1275–1285
56. Wiest R, Rath HC (2003) Gastrointestinal disorders of the critically ill. Bacterial translocation in the gut. *Best Pract Res Clin Gastroenterol* 17:397–425
57. Desai MH, Herndon DN, Rutan RL, Abston S, Linares HA (1991) Ischemic intestinal complications in patients with burns. *Surg Gynecol Obstet* 172:257–261
58. Rogler G (2004) Update in inflammatory bowel disease pathogenesis. *Curr Opin Gastroenterol* 20:311–317
59. Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E (2004) Genetic polymorphisms predicting the outcome of bone marrow transplants. *Br J Haematol* 127:479–490